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The effectiveness of seed coating with microbial fungicide on controlling seed quality and damping-off disease of tomato

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Abstract Microbial seed coating was not significantly influenced germination percentage, germination index, or germination speed under laboratory conditions. Whereas, seeds coated with *Trichoderma asperellum* and *Bacillus subtilis* consistently demonstrated to improve seedling emergence and vigor in greenhouse conditions. With respect to disease suppression, coatings seeds with *T. asperellum* resulted to reduce both the incidence and severity of damping-off disease caused by *Pythium torulosum.* The lowest disease incidence (30.50%) and severity (17.43%) were observed in seedlings from *T. asperellum*-coated seeds. These findings highlighted the potential of antagonistic microbial coatings to promote seedling health and provided a sustainable strategy for managing soilborne diseases. This approach is offered a promising complement or alternative to chemical control methods in commercial tomato seedling production.

Keywords: Seed treatment, Seed germination, *Trichoderma asperellum*, *Bacillus subtilis*, *Pythium* sp., Damping-off

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an economically important crop in Thailand and constitutes a major hub for tropical seed production worldwide. Among vegetable seeds, tomato seeds rank highest in export value. In 2022, exports reached 37,571 kg, valued at approximately 1,400 million THB (Thai Seed Trade Association, 2023). Thailand exports tomato seed to several countries, including the United States, Japan, and the Netherlands (Department of Agriculture, 2020). However, cultivation is restricted to specific production seasons (Department of Agricultural Extension, 2020). Tomatoes perform best in the cool season, whereas the hot and rainy seasons are unfavorable. High humidity during the rainy season increases the incidence of foliar and root diseases, reduces yields, and heightens susceptibility to insect pests and

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pathogens (Phrommarin, 2017). Major tomato diseases include damping-off, late blight, and powdery mildew (Department of Agriculture, 2020a,b). Root-related diseases are particularly destructive during the seedling stage, with root rot caused by *Pythium* spp., *Phytophthora* spp., and *Fusarium* spp., resulting in considerable economic losses.

Damping-off, caused primarily by *Pythium* spp., infects roots and stem bases at all growth stages, resulting in seed rot, pre- and post-emergence damping-off, root and stem rot, and seedling wilt (Robertson, 2012; Jenkins and Averre, 1983). Outbreaks are most severe during the hot and humid rainy season, when the pathogen proliferates rapidly (Chamswarng and Gesnara, 1988). Effective management is challenging because the pathogen persists in soil, and chemical treatments applied at the seedling stage are often ineffective once infection is established (Promchote *et al*., 2014).

Seed coating is a promising strategy that enables uniform application and strong adherence of protective agents, thereby improving seed and seedling quality (Siri, 2015). However, chemical coatings may pose health and environmental risks, as well as leave pesticide residues in produce. Consequently, microbial bioproducts are increasingly being investigated as sustainable alternatives. Plant beneficial microorganisms (PBM) can enhance plant growth and suppress pathogens, including plant growth-promoting fungi (PGPF) such as *Trichoderma asperellum*, a soil-borne fungus with strong antagonistic activity, and plant growth-promoting bacteria (PGPB) such as *Bacillus subtilis*, which has been reported to inhibit *Pythium* mycelial growth (Chamswarng and Intanoo, 1999; Paravar *et al*., 2023). These microorganisms promote plant health through multiple mechanisms, including phytohormone production, induced systemic resistance, and secretion of defensive enzymes (Souza *et al*., 2015; Adedayo and Babalola, 2023).

Biological control using microbial agents represents a sustainable alternative to synthetic chemicals for the management of soilborne pathogens. Therefore, this study aimed to evaluate the quality of tomato seeds coated with antagonistic microbial bioproducts and to assess their effectiveness in controlling damping-off disease in tomato seedlings.

Materials and methods

Experimental design

The experiment was conducted at the Seed Technology Laboratory, Plant Pathology Laboratory, and greenhouse facilities of King Mongkut's Institute of Technology Ladkrabang, Thailand. A completely randomized design (CRD) with four replications was employed. The treatments consisted of: (i) uncoated

seeds (control), (ii) seeds coated with hydroxypropyl methylcellulose (HPMC), (iii) seeds coated with *Trichoderma asperellum*, and (iv) seeds coated with *Bacillus subtilis*.

Isolation and preparation of antagonistic microorganisms

Trichoderma asperellum was isolated from bioproduct of Kasetsart University Kamphaeng Saen Campus and cultured on potato dextrose agar (PDA) for 7 days at 28°C. Identification was carried out based on colony morphology and microscopic characteristics. *Bacillus subtilis* was obtained from a commercial bioproduct and maintained on nutrient agar (NA) for 24 hr at 28°C. Fresh inocula was prepared prior to seed coating to ensure viability.

***Isolation of Pythium* sp.**

Pythium torulosum was obtained from the Plant Pathology Laboratory, Shcool of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang. (Maiprom *et al*., 2023)

Seed preparation and microbial suspensions

Tomato seeds were surface-sterilized with 10%(v/v) sodium hypochlorite (Clorox®) solution for 5 min, rinsed three times with sterile water, and air-dried in a laminar flow hood. *T. asperellum* spore suspension (10^6 spores/mL) was prepared from 7-days-old PDA cultures, *B. subtilis* suspension (10^{10} cells/mL) was obtained from NA/NB cultures grown at 150 rpm for 24 hr and adjusted to the desired concentration by optical density at 600 nm (OD_{600}) in normal saline.

Seed coating

Hydroxypropyl methylcellulose (HPMC, 1%) was prepared by dissolving 1 g in 100 mL of distilled water and subsequently mixed with 25 mL each of *T. asperellum* and *B. subtilis* suspensions. Seeds were coated using a rotary coater (RRC150) at a rate of 180 mL coating solution per kg of seed (Thiphinkong, 2022), air-dried at room temperature for 48 hr, and subsequently divided for viability, quality, and disease control tests.

***In vitro antagonism against Pythium* sp.**

Dual culture assays were conducted to assess antagonistic activity. For *T. asperellum*, PDA plugs of *P. torulosum* and *T. asperellum* were placed opposite

each other, 2 cm from the plate edge. For *B. subtilis*, *P. torulosum* plugs were placed centrally on PDA, with bacterial streaks 2 cm from each side. Plates were incubated at room temperature for 4 days (Prasom, 2018), and the mycelial pathogen was measured at 24, 48, 72, and 96 hr to calculate percent inhibition.

$$\text{inhibition (\%)} = \left[\frac{\text{R1-R2}}{\text{R1}}\right] \text{x } 100$$

Where:

- R1 = mean mycelial radius of the pathogen in the control plates
- R2 = mean mycelial radius of the pathogen in the treatment plates

This formula was used to calculate the reduction in pathogen growth relative to the untreated control.

Viability of microorganisms on coated seeds

Fifty coated seeds per treatment were plated on PDA (*T. asperellum*) or NA (*B. subtilis*) with five replications of 10 seeds each. After incubation at room temperature for 48 hr (Phutthang, 2010). Colony growth was recorded, and viability was expressed as the percentage of seeds using the following formula:

$$\text{Viability (\%)} = \left[\frac{\text{Number of seeds showing fungi growthtotal seed}}{\text{Total number of seedling}}\right] \text{x } 100$$

Seed germination under laboratory (SGL)

Germination tests were conducted using the top-of-paper method with four replications of 50 seeds per treatment. Seeds were incubated under controlled conditions in a germination chamber maintained at a constant temperature of 25°C. The number of normal seedlings was recorded on the 5th day (first count) and on the 14th day (final count), following the standard procedure outlined by ISTA (2019). Germination percentage was calculated using the following formula:

$$\text{Seed germination (\%)} = \left[\frac{\text{Number of normal seedlings}}{\text{Total number of seeds}}\right] \text{x } 100$$

Seed germination under greenhouse (SGG)

For each treatment, 50 seeds were randomly selected per replicate, with four replicates, and sown in seedling trays filled with peat moss under greenhouse conditions. The temperature was maintained at 28–35 °C, and the trays were watered once daily. Normal seedlings were recorded at 5 days after sowing (first count) and at 14 days after sowing (final count), following ISTA (2019) guidelines. Germination percentage was subsequently calculated.

Germination index (GI)

For the germination index tests, the number of normal seedlings was recorded on the 5th day (first count) and on the 14th day (final count), following the standard procedure outlined by ISTA (2019). The germination index was calculated using the following formula:

$$\text{Germination index (\%)} = \Sigma \left[\frac{\text{Number of normal seedling in each days}}{\text{Number of day after cultivation}} \right]$$

Seedling root length (RL)

From each replication of the germination test, ten seedlings were randomly selected. The root length of each seedling was measured using a ruler, from the junction of the root and shoot to the root tip. The mean root length was then calculated using the following formula:

$$\text{Mean of root length (cm)} = \left[\frac{\text{Sum of seedlings root length}}{\text{Number of seedlings}} \right]$$

Seedling shoot length (SL)

Shoot length was measured using a ruler, from the root-shoot junction to the apical meristem:

$$\text{Mean of root length (cm)} = \left[\frac{\text{Sum of seedlings shoot length}}{\text{Number of seedlings}} \right]$$

Seedling dry weight (DW)

Seed vigor was assessed using seedling dry weight as a criterion, as vigorous seeds typically produce seedlings with higher dry weight. Seedlings from the root and shoot length test were dried in a hot-air oven at 80°C for 24 hr. The mean dry weight was then calculated using the following formula:

$$\text{Mean of dry weight (mg/plants)} = \left[\frac{\text{Sum of seedlings dry weight}}{\text{Number of seedlings}} \right]$$

Speed of germination (SG)

The speed of Germination tests was conducted by recording the number of normal seedlings on the 5th day (first count) and on the 14th day (final count), following the standard procedure outlined by ISTA (2019). The speed of Germination was then calculated using the following formula:

$$\text{Speed of Germination (seedling/day)} = \Sigma \left[\frac{\text{number of noemal seedling in each days}}{\text{number of day aftercultivation}} \right]$$

Efficacy of tomato seeds coated with microbial products to control of Damping-off

Tomato seeds, both coated and uncoated with various microbial product treatments, were sown in sterilized peat moss using 200-cell seedling trays. Two soil conditions were established: (i) soil inoculated with *P. torulosum* at 10^6 propagules/mL, applied at 100 mL per kg of growing medium (Jaikengkaj, 2017), and (ii) pathogen-free soil as a control. Each treatment was replicated four times with 50 seeds per replicate. The following parameters were evaluated:

Seed survival

Seedlings were observed 14 days after sowing. Emergence was confirmed when the roots protruded from the seed coat. Seed survival (%) was calculated using the following formula:

$$\text{Seed survival (\%)} = \left[\frac{\text{Number of germinated seeds}}{\text{Number of seed tested}} \right] \text{x } 100$$

Disease incidence (DI)

Seedlings exhibiting damping-off symptoms were recorded 14 days after sowing. Disease incidence (%) was calculated using the following formula:

$$\text{Disease incidence (\%)} = \left[\frac{\text{Number of diseased seedlings}}{\text{Number of seedling tested}} \right] \text{x } 100$$

Disease severity (DS)

Symptoms of seedling wilt were recorded 14 days after sowing. Disease severity was assessed according to Wong *et al*. (1984) using a 0–5 rating score:

0 = healthy, no symptoms
1 = <25% roots red
2 = 25–49% roots brown lesions
3 = 50–74% roots brown rot
4 = ≥75% roots affected, seedlings wilted and base damaged
5 = dead seedlings, complete root rot

Disease severity (%) was calculated using the formula:

$$DS\ (\%) = \left[\frac{\Sigma\ (\text{number of seedlings x severity rating})}{\text{Total number of seedling x maximum severity rating}}\right] x\ 100$$

Post-emergence seedling damping-off

Seedling mortality (%) was determined from the number of seedlings rated at severity level 5, and was calculated using the following formula:

$$Die\ (\%) = \left[\frac{\text{Number of dead seedling (level 5)}}{\text{Number of seedling tested}}\right] x\ 100$$

Statistical analysis

All recorded data were subjected to analysis of variance (ANOVA) following a completely randomized design (CRD) using the Statistical Analysis System (SAS). Treatment means were separated using the Least Significant Difference (LSD) test.

Results

Colony of *T. asperellum* on PDA were initially yellowish-green and became dark green with age. The mycelia formed loose concentric rings, and abundant conidia aggregated into clusters resembling fine sand particles (Figure 1). Colony of *B. subtilis* on NA were rod-shaped, Gram-positive, and capable of forming spores and capsules (Figure 2).

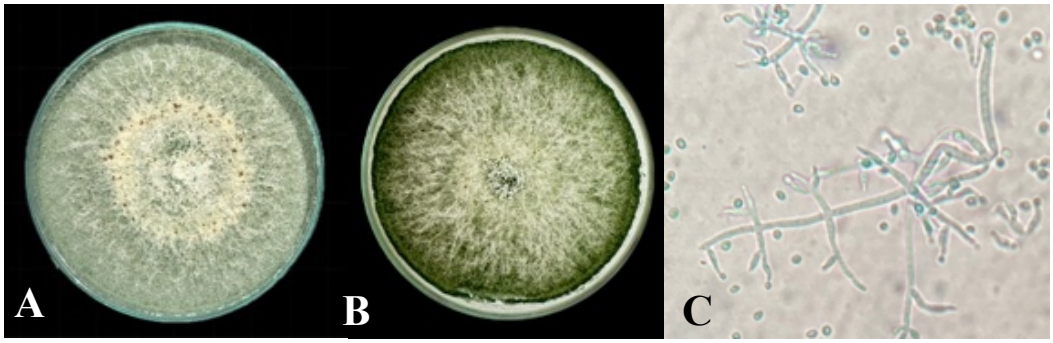

Figure 1. Morphology of *T. asperellum* on PDA medium at 7 days (A) and 14 days (B), and spores with hyphae observed under a light microscope at 400x magnification (C)

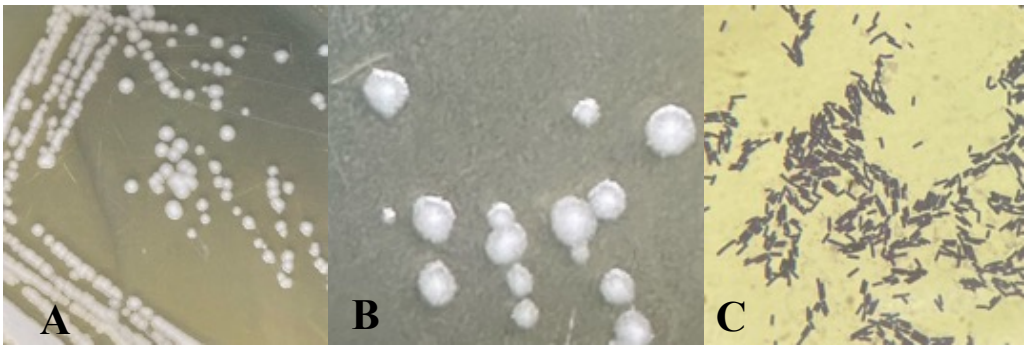

Figure 2. Morphology of *B. subtilis* on NA medium at 2 day(A), single-colony appearance (B), and cell morphology observed under a light microscope at 1000x magnification (C)

Efficacy of T. asperellum and B. subtilis against P. torulosum in vitro

Microbial products significantly suppressed the colony growth of *P. torulosum*. *T. asperellum* which exhibited strong inhibitory activity, colony diameters were 4.53, 4.65, and 5.62 cm after 48, 72, and 96 hr, corresponding to inhibition rates of 4.89%, 25.51%, and 27.28%, respectively (Table 1; Figure 3A). In contrast, *B. subtilis* showed only a slight effect, reducing colony diameter to 4.33 cm at 48 hr, with inhibition ranging from 1.63 to 5.10%, which was not statistically different from the control (Table 1; Figure 3B). Overall, these results demonstrated that *T. asperellum* was more effective than *B. subtilis* in suppressing *P. torulosum* under laboratory conditions.

Table 1. Effectiveness of *T. asperellum* and *B. subtilis* in inhibits mycelial growth of *P. torulosum* at 24, 48, 72, and 96 hours after dual culture test

Treatment	***Pythium torulosum***							
	Colony (cm)				% inhibition of colony			
	24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr
Control	2.15	4.77^{a}	6.25^{a}	7.73^{a}	-	-	-	-
T. asperellum	2.03	4.53^{b}	4.65^{b}	5.62^{b}	5.36	4.89^{a}	25.51^{a}	27.28^{a}
B. subtilis	2.03	4.33^{c}	6.03^{a}	8.12^{a}	3.91	5.10^{a}	3.70^{b}	1.63^{b}
LSD (*P*=0.01)	ns	**	**	**	ns	*	**	**
C.V. (%)	3.01	1.42	4.29	3.52	81.66	48.35	39.47	34.76

ns; not significantly different * and ** significantly different at 95% and 99%.
$^{a-c}$ Different superscript letters within each row are significantly different (P<0.01) by method of Least significant difference (LSD).

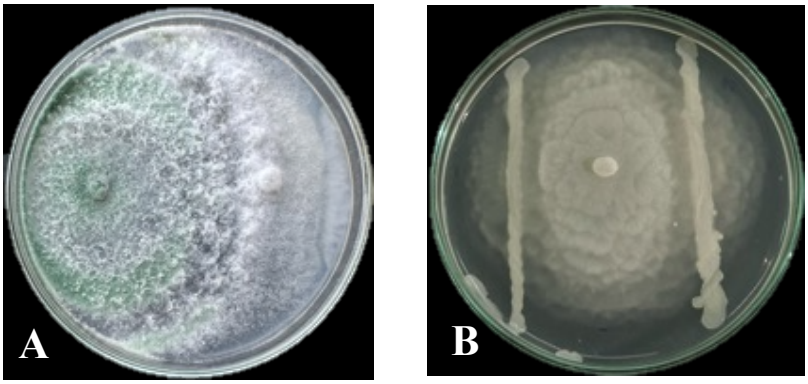

Figure 3. Efficiency of *T. asperellum* (A) and *B. subtilis* (B) in inhibit *P. torulosum* in a dual culture test on PDA medium at 4 days of incubation

Viability of microorganisms on seed surfaces after coating

Seed coating was performed using hydroxypropyl methylcellulose (HPMC) as an adhesive. The results indicated that microorganisms adhered well to the seed surface and remained viable after coating. Seeds coated with *T. asperellum* exhibited 100% microbial survival (Figure 4A), whereas those coated with *B. subtilis* showed 96% survival (Figure 4B). The study demonstrated that HPMC is found to be suitable for coating *B. subtilis* without reducing microbial viability.

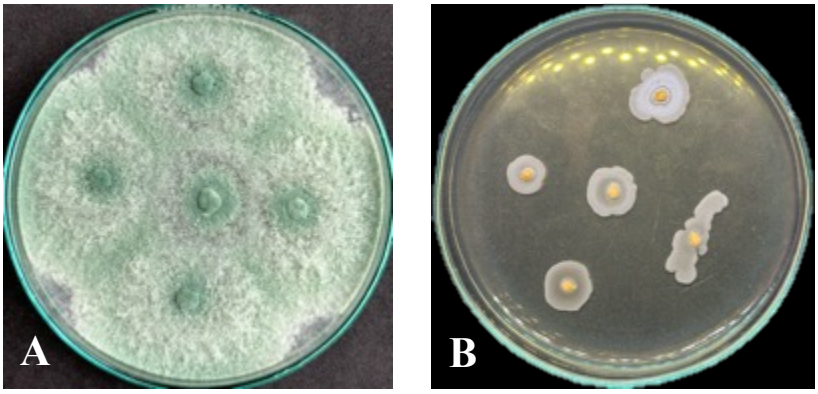

Figure 4. Viability of *T. asperellum* on PDA medium (A) and *B. subtilis* on NA medium (B) after seed coating

Seed quality and seedling growth

In vitro germination of tomato seeds was not significantly affected by coating with antagonistic microorganisms (*T. asperellum* or *B. subtilis*) or with

HPMC, indicating that microbial coatings did not compromise seed viability (Table 2). Germination index and speed of germination were likewise unaffected, although seeds coated with *T. asperellum* and *B. subtilis* tended to exhibit slightly higher germination indices than controls.

Coating treatments significantly affected seedling growth ($p < 0.01$). Seeds coated with *B. subtilis* produced the longest roots (7.23 cm), followed by those coated with HPMC (5.00 cm), whereas seeds coated with *T. asperellum* produced the shortest roots (3.96 cm). In contrast, shoot length was greatest in HPMC-coated seedlings (9.12 cm) and lowest in *B. subtilis*-coated seedlings (4.47 cm), with *T. asperellum*-coated seedlings measuring 8.02 cm. Seedling dry weight was not significantly influenced by any coating treatment (Table 2).

Under greenhouse conditions with pathogen-free soil, germination rates did not differ significantly among treatments. However, seeds coated with *T. asperellum* and *B. subtilis* exhibited higher seedling survival rates (91% and 89%, respectively) than the other treatments (Table 2).

Table 2. Seed germination, Germination index, Speed of germination, Root length, Shoot length, and Seedling dry weight of tomato seedlings under laboratory conditions

Treatment	SGL (%)	GI	SG (seedling/day)	RL[1/] (cm)	SL[1/] (cm)	DW (mg/seedling)
T1	92.00	7.66	8.70	4.68^{bc}	8.83^{a}	1.66
T2	83.50	7.23	7.86	5.00^{b}	9.12^{a}	2.09
T3	93.00	9.01	8.45	3.96^{c}	8.02^{b}	1.91
T4	90.00	8.20	8.58	7.23^{a}	4.47^{c}	1.80
LSD (P=0.01)	ns	ns	ns	**	**	ns
C.V. (%)	4.90	10.05	6.43	10.72	5.66	11.54

T1 = Uncoated seeds; T2 = HPMC coated seed; T3 = *T. asperellum* coated seed; T4 = *B. subtilis* coated seed

SGL= Seed germination under labolatory conditions; GI = Germination index; SG = Speed of germination; RL = Seedling root length; SL = Seedling shoot length; DW = Seedling dry weight. ns; not significantly different. ** significantly different at 99%.

[1/a-c] Different superscript letters within each row are significantly different (P<0.01) by method of Least significant difference (LSD).

Seed survival

Seed coating did not significantly affect seedling survival in pathogen-infested soil. However, coated seeds tended to exhibit higher survival rates compared with uncoated seeds. Notably, seeds coated with *T. asperellum* achieved the highest survival rate (91%) (Table 3).

Disease incidence

Coating with antagonistic microorganisms significantly affected damping-off incidence ($p < 0.05$). Uncoated seeds exhibited the highest disease incidence (54%), which was not significantly different from seed coated with HPMC-coated (47%) and *B. subtilis*-coated seeds (39.5%). In contrast, seeds coated with *T. asperellum* showed the lowest incidence (30.5%) (Table 4).

Table 3. Germination under greenhouse conditions (pathogen-free soil) and seed survival in pathogens- infested soil

Treatment	SGG (%)	seed survival (%)
T1	75.50	70.50
T2	70.50	80.50
T3	78.50	91.00
T4	89.00	86.00
LSD	ns	ns
C.V. (%)	10.91	14.68

T1 = Uncoated seeds; T2 = HPMC coated seed; T3 = *T. asperellum* coated seed; T4 = *B. subtilis* coated seed, SGG = Seed germination under greenhouse conditions, ns; not significantly different.

Disease severity

No significant differences in disease severity were observed among treatments. However, seeds coated with *T. asperellum* tended to exhibit lower severity (17.43%) compared with the other treatments (Table 4).

Seedling death

Seedling mortality was highest in uncoated seeds (15%), followed by seeds coated with HPMC (10%). Seeds coated with *T. asperellum* and *B. subtilis* showed the lowest mortality rates at 8.5% and 8%, respectively (Table 4).

Table 4. Disease incidence, disease severity and seedling mortality (%) in soil inoculated with pathogens

Treatment	DI[1/] (%)	DS (%)	seedling death (%)
T1	54.00 a	29.14	15.00
T2	47.00 a	24.68	10.00
T3	30.50 b	17.43	8.50
T4	39.50 ab	22.16	8.00
LSD (*P*=0.05)	*	ns	ns
C.V. (%)	22.29	36.09	78.25

T1 = Uncoated seeds; T2 = HPMC coated seed; T3 = *T. asperellum* coated seed; T4 = *B. subtilis* coated seed, DI = Disease incidence; DS = Disease severity, ns; not significantly different and * significantly different at 95%, [1/a-b] Different superscript letters within each row are significantly different ($P<0.05$) by method of Least significant difference (LSD).

Discussion

In vitro dual culture tests showed that *T. asperellum* and *B. subtilis* significantly reduced the colony growth of *P. torulosum*, with the strongest inhibition observed after 48 hr (4.53 cm and 4.33 cm, respectively). *T. asperellum* exhibited greater inhibition than *B. subtilis*, confirming its superior antagonistic potential, these findings are consistent with previous reports on *Trichoderma* species suppressing oomycete pathogens, including *P. torulosum*. Similarly, Pimente *et al*. (2022) demonstrated that various *Trichoderma* species suppressed *Pythium aphanidermatum* and penetrated pathogen colonies.

Seed coating with HPMC enabled strong microbial adhesion and survival, with *T. asperellum* and *B. subtilis* maintaining 100% and 96% viability, respectively. Coating did not significantly affect seed germination, germination index, seedling growth, or dry weight, although microbial treatments tended to improve greenhouse germination and enhance germination uniformity. Notably, coating the seeds with *B. subtilis* increased the root length. These findings are consistent with Phutthang (2010), who reported that 1% hydroxyethyl cellulose (HEC) had no effect on the growth of *Trichoderma harzianum*. Our results confirm that HPMC, another cellulose-based polymer, is highly compatible with both *T. asperellum* and *B. subtilis*. Its ability to form a protective, biodegradable film enhances microbial adhesion and preserves cell viability, while its non-toxic and non-inhibitory nature ensures microbial survival on the seed surface and effective release during germination. Sujatha *et al*. (2023) similarly reported that polymer function as protective barriers for biological agents, extending shelf life while facilitating microbial colonization of seedling rhizosphere to protection against soil-borne pathogens. Such polymer-based coating technology improves germination uniformity and represents a cost-effective, time-efficient, and practical alternative to chemical-based plant protection and management.

Microbial coatings improved the management of damping-off disease. Seeds coated with *T. asperellum* and *B. subtilis* exhibited higher survival (91% and 86%), lower disease incidence (30.5% for *T. asperellum*), and reduced severity (17.43% for *T. asperellum*) compared with the controls. These results are consistent with previous studies demonstrating that *Trichoderma* and *Bacillus* species can effectively control soil-borne pathogens and enhance seedling survival. Overall, Trichoderma-based coatings offer a promising biocontrol strategy for sustainable disease management in crops. These findings corroborate earlier reports (Kipngeno *et al*., 2015; Pimente *et al*., 2022) that coating seeds with *Trichoderma* or *Bacillus* species can effectively reduce

damping-off caused by *Pythium* spp. and improve seedling survival. Furthermore, Liu *et al*. (2022) reported variable control efficiencies of *Trichoderma* species against cucumber damping-off, with *T. koningiopsis* and *T. gamsii* demonstrating superior performance (80.33% and 82.67%) compared with *T. asperellum* (35.49%). These strains exhibited strong antagonism against oomycete pathogens and can be integrated into effective biocontrol strategies, highlighting the potential of microbial seed coatings for sustainable disease management in crops. Chandrika *et al*. (2024) developed chitosan-cellulose biopolymer matrices containing *T. harzianum* (Th4d), which achieving substantial disease reduction in multiple crops: 64.7% in marigold, 72% in peanut, and 70.9% in soybean. Complementing these findings, Xie and Yang (2023) demonstrated that *Trichoderma guizhouense* NJAU4742 seed coating not only improved germination and plant growth but also enhanced soil enzyme activities while preserving beneficial microbial diversity in the rhizosphere.

Coating tomato seeds with the antagonistic microbes *T. asperellum* and *B. subtilis* did not significantly affect seed germination, germination index, or seedling dry weight, although a trend toward improved germination under greenhouse conditions was observed. *T. asperellum* reduced root length, whereas *B. subtilis* slightly reduced shoot length. Importantly, both microbes tended to enhance seedling survival and suppress damping-off caused by *Pythium* spp., with *T. asperellum* showing the lowest disease incidence (30.5%) and severity (17.43%). Overall, these findings highlighted the potential of coating seeds with plant-beneficial microorganisms as an effective method to improve seed quality and manage tomato damping-off disease.

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Conflicts of interest

The authors declare no conflict of interest.

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